RNA Structure: Roles of Me^{2+}

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Divalent metal ions play an important role in charge neutralization and incorrectly folding RNA into its three-dimensional structure. They also play a potentially important role in the catalytic activity of RNA.

Secondary article

Article Contents

Introduction

The ability of RNA to fold into complex tertiary structures allows it to perform structural and functional roles in biological processes. In order to allow the negatively charged phosphodiester backbones to pack closely, their charges must be neutralized in some way. The main candidates for this charge neutralization are the positively charged metal ions. While monovalent metal ions are clearly important in this regard, the divalent metal ions have two important advantages; their charge density is higher, allowing more effective charge neutralization, and they have specific coordination geometries, favouring structural complementarity in the RNA environment.

Inside cells, the metal ion concentrations are adjusted and maintained by a variety of active and passive transport systems. Often large amounts of metal ions are bound to intracellular macromolecules. **Table 1** shows the concentrations of divalent metal ions and also lists some of their physical properties.

In addition to the ions listed in **Table 1**, Be, Ra, Pt, Cu and Hg can exist in the divalent state. Most of the divalent metal ions have a coordination number of six, i.e. they can form hexacoordinate complexes with six ligand molecules at the apexes of an octahedron. Although generally octahedral, Ca^{2+} and transition metals are flexible in both coordination number and geometry.

Divalent metal ions can bind to nucleic acids via a number of mechanisms. The divalent metal ions can bind through phosphate oxygen to form salt-like complexes between positively charged metal ions and negatively charged phosphate oxygens. They can also bind directly to the bases. In pyrimidines, this is via the O2 (cytidine) or the O2, O4 (uridine). In the purines, binding usually occurs through N7. The O6 can accept H-bonds from ligands in the coordination sphere of the divalent metal ion and these interligand interactions contribute to complex formation. Some divalent metal ions (Mg, Ca, Ba, Cd) can also interact with the sugar hydroxyls. The hydroxyl group enters the metal ion coordination sphere with the lone electron pair of the oxygen. Often, water molecules can complete the coordination sphere.

For a given RNA, such as transfer RNA (tRNA), there may be a large number of low-to medium-affinity binding sites ($K_d = 10^{-3}$–10^{-4}) and a small number (i.e. 5–10)

Table 1 Physical properties of divalent metal ions

Metal	Coordination number	pK_a of metal hydrate	pH at 10 mmol L^{-1} metal ion concentration	Concentration in bacterial cell (mg kg^{-1})
Mg	6	11.4	9.6	7000 (~3 mmol L^{-1} intracellular)
Ca	6, 8	12.9	12.4	5100
Ni	6	9.9	8.1	4600
Mn	6	10.6	7.9	260
Zn	4, 6	9.0	6.6	83
Co	6	10.2	7.2	7.9
Cd	4–7	9.6	8.1	0.31
Pb	4–7	7.7	7.2	–
Ba	6, 7	13.5	–	–
Sr	6	13.2	–	–

high-affinity binding sites ($K_d = 10^{-5}$–10^{-6}) for a specific divalent metal ion. The low-affinity binding sites are usually described as purely electrostatic in nature and are in rapid exchange with other ions in solution. The high-affinity, slow exchanging sites are localized as determined in crystal structures and bind through formation of coordination complexes of both the metal and its hydration sphere. These specifically bound divalent metal ions are thought to be critical for stabilization of RNA structure and possibly in nucleation of the folding process. An unresolved question is whether divalent metal ion binding can induce conformational change in RNA or whether binding occurs at preformed sites in the RNA. A further outstanding question is the role of divalent cations in ribozyme catalysis. With these questions in mind, we will look at the known structures of RNA with associated divalent metal ions bound.

Structures of RNA with Bound Divalent Cations

The three-dimensional structures of RNA with bound divalent cations are our best source of information about their specificity and geometry of binding; their role in RNA folding and stabilization of RNA structure; and their potential catalytic function. Detailed structural information about RNA–metal complexes comes from X-ray crystallography and high-resolution multidimensional NMR studies. **Table 2** summarizes the structurally characterized RNA–divalent metal ion complexes currently stored in the Nucleic Acid Database (NDB) and the Protein Data Bank (PDB). A detailed description of the specific divalent cation binding to transfer RNA and pseudoknots as determined by structural studies is given as examples of the type of information these studies provide.

Transfer RNA

The crystal structure of tRNA was solved at 2.5–2.7 Å resolution in two different forms in 1974 (Kim *et al*., 1974; Robertus *et al*., 1974). At the time, five high-occupancy magnesium hydrate-binding sites were identified. A recent high-resolution structure, at 1.9 Å resolution (Shi and Moore, 2000), reveals several new binding sites and confirms details under dispute in the original structures. In total, 11 specific divalent metal-binding sites are seen (**Figure 1**), seven of which bind Mg^{2+} (and sometimes other metals), two bind Mn^{2+} exclusively and another two bind only Co^{2+}. One site, M3, in the hinge region, was seen to bind all three cations. Another site, in the same region, binds both Mg^{2+} and Mn^{2+}. The metal ions are all

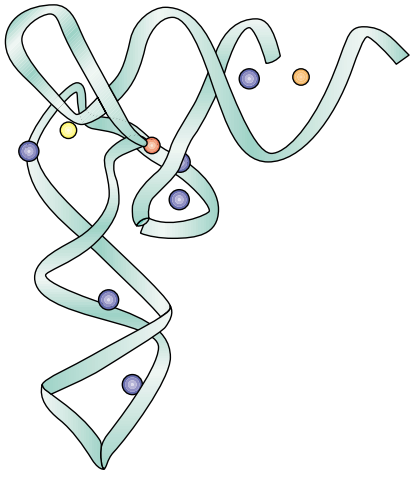

Figure 1 The high-resolution crystal structure of transfer RNA and bound divalent metal ions. The sites that can bind magnesium are shown in blue, the site that can bind cobalt or magnesium is shown in orange, the site that can bind magnesium, manganese or cobalt is shown in magenta and the site that binds magnesium, manganese, zinc, cobalt or lead is shown in yellow.

Table 2 Crystal structures of RNA–divalent metal ion complexes found in the nucleic acid database

Me^{2+}	Number of examples	Principal mode of binding	Notable examples
Mg	18	Phosphate backbone	Hammerhead and leadzyme (ribozymes), sarcin-ricin, 5S (ribosomal RNA), P4-P6 (group I intron domain), tRNA, pseudoknot
Ca	5	Phosphate backbone	Pseudoknot, TAR (element of HIV-1)
Sr	4	Phosphate backbone	
Mn	3	Guanine base	Hammerhead, tRNA
Co	1	Guanine base	Hammerhead, tRNA
Hg	1	G•U wobble pair	5S rRNA
Cd	1	Guanine base	

octahedrally coordinated, except for a Mg^{2+} binding in the anticodon loop. A change in the geometry of binding is seen for the site that binds the three cations depending upon which metal is bound. Five of the Mg^{2+} sites were those found in the original structures. Biochemical evidence has also shown that M3 is also capable of binding Zn^{2+}. It appears that for some but not all, high-affinity sites, a variety of divalent metal ions will be able to bind and perform their role of charge neutralization. The manner in which they do so and their effect upon the surrounding structure will depend upon the properties of the particular ion.

Pseudoknots

RNA pseudoknots are a classic family of tertiary structures formed when nucleotides in the loop of a hairpin pair with nucleotides from a single-stranded region, forming a second strand and loop. Pseudoknots have many different functional roles in biological systems. They occur at the 3′-termini of several plant virus RNA genomes, in RNase P RNA, within 16S RNA and also in ribozymes. They are involved in translational control and can cause ribosomal frameshifting in retroviruses.

The solution structure of the VPK pseudoknot from mouse mammalian tumour virus with the Mg^{2+} hexahydrate analogue $Co(NH_3)_6^{3+}$ was recently determined by NMR methods (Gonzales and Tinoco, 1999). The $Co(NH_3)_6^{3+}$ binds at the sharp loop-to-stem turn and reduces electrostatic repulsion of the phosphates in the three proximal strands. It was proposed that binding of a divalent metal ion in this position is a general feature of all frameshifting pseudoknots regardless of sequence. All that is required in these pseudoknots is a one to two nucleotide loop, a five to seven nucleotide base-paired stem and the availability of a number of nonspecific hydrogen bond acceptors. It appears that Mg^{2+} is required for correct folding of this class of pseudoknot, in order to stabilize the sharp turn in the molecule. The binding energy is provided by electrostatic attraction to the phosphates and water-mediated hydrogen bonds to acceptors on the backbone and bases of the RNA.

A crystal structure of a pseudoknot-containing aptamer selected for its biotin-binding ability revealed six hydrated Mg^{2+} ions. Four of the six metal ions bind in the major groove of the double helix. One Mg^{2+} ion, however, was seen in the sharp turn described above and also made additional contacts that facilitated biotin binding. The final Mg^{2+} lies at the junction between two helices in the major groove and is directly coordinated to one of the bases.

Catalytic RNA

Ribozymes are RNA molecules that can function as catalysts. At least eight different catalytic RNAs (ribozymes) occur in nature and all catalyse phosphoryl transfer reactions. The rate of phosphoryl transfer can be accelerated by numerous factors, including stabilization of unfavourable charge development in the transition state, positioning of atoms and ground-state destabilization. All of these can be influenced by divalent metal ions. In RNA, adenosine and cytosine have the potential for protonation of their ring nitrogens N1 and N3 respectively, but the pK_a values for the free nuclosides are too low. Perturbation of adenosine and cytosine has been observed, which suggests that effective acid–base catalysis may be possible in RNA.

The range of chemical reactions that are catalysed by ribozymes is relatively modest. This is mainly because the number of functional groups that can participate in the chemistry is limited compared with the set of amino acid side-chains. The great majority of reactions carried out by ribozymes are transesterification reactions.

Divalent metal ions can participate in the reactions in a number of ways. First, it may help the RNA fold into a conformation that achieves a pre-activation of the phosphodiester bond. All the ribozymes have interesting secondary structural features. Secondly, divalent metal ions can play a more direct role in the chemistry of phosphodiester bond cleavage. It has been proposed that divalent metal ions can play a catalytic role in one of four ways. They can act as either a Lewis acid to stabilize the transition state and/or facilitate the departure of the oxyanion leaving group. A hydroxide ion in the coordination sphere of a divalent metal ion could act as a general base to increase the nucleophillicity of the 2′-hydroxyl. They may play a role in the activation of the leaving group or they may help to activate a nucleophile (**Figure 2**).

Until recently, all group I ribozymes, and possibly all naturally occurring ribozymes, were thought to be metalloenzymes, utilizing bound divalent cations for catalysis. However, three of the four small ribozymes (hammerhead, hairpin and *Neurospora* VS ribozymes) have been shown to function in the presence of high concentrations of monovalent ions alone. The hammerhead and hairpin ribozymes are two small self-cleaving RNAs that mediate rolling-circle replication in small single-stranded circular virus and virus-like RNAs that infect plants. The VS ribozyme is derived from a self-cleaving RNA motif that mediates rolling circle replication of the Varkud plasmid of *Neurospora*. The hepatitis delta virus (HDV) ribozyme is a satellite RNA virus that is occasionally associated with hepatitis B, mediates rolling-circle replication of delta virus. These are described below.

Hammerhead ribozyme

The structure of the hammerhead ribozyme as revealed by X-ray crystallography (Scott *et al*., 1996) has an Mg^{2+} ion binding directly to the pro-R phosphate oxygen adjacent to

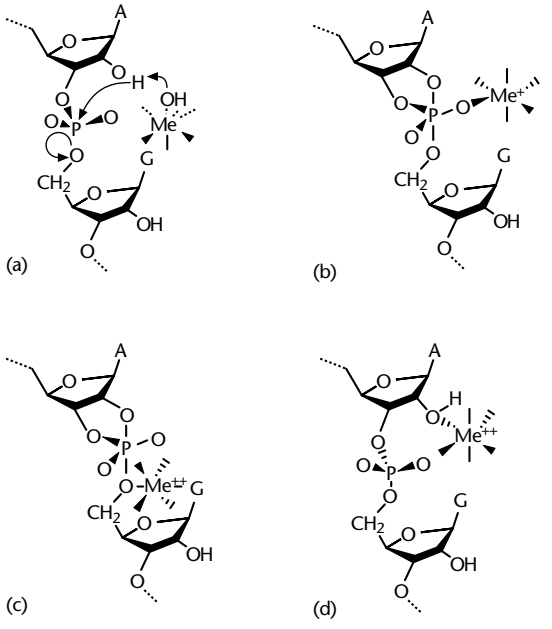

Figure 2 The four possible modes of RNA catalysis using divalent metal ions. (a) General base catalysis; (b) Lewis acid (electrophilic) catalysis; (c) leaving group activation; (d) nucleophilic activation.

the scissile bond. Further metal-binding sites have been identified by nuclear magnetic resonance (NMR) spectroscopy to be in the hairpin loop and also helix II. The single-ion hypothesis assumes that a metal–hydroxide complex ion binds to the pro-R phosphate oxygen and deprotonates the 2′-OH group. This is followed by an attack by the 2′-oxyanion on the phosphorous atom and subsequent departure of the 5′-nucleoside. A different mechanism, the two-metal-ion model, associates an ion with the 2′-hydroxyl group of the attacking ribonucleoside and the other with the 5′-hydroxyl group of the departing nucleoside. Both are consistent with the existing evidence.

Hairpin

The role of the metal ion cofactor in the reaction catalysed by the hairpin ribozyme is that it is required for structural purposes (Young *et al*., 1997). It is accepted that catalysis requires the association of loops 1 and 2. The association of the looped areas of the RNA molecule must overcome the charge repulsion of the phosphodiester backbone; metal ions could assist in facilitating this. Mg^{2+}, Sr^{2+} and Ca^{2+} are all capable of acting as the cofactor in the hairpin ribozyme reaction, although the fastest reaction rates are observed with Mg^{2+}. However, other divalent metal ions such as Mn^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+} and Ba^{2+} are incapable of supporting catalysis, although reaction is observed with Mn^{2+} and Co^{2+} in the presence of spermidine. Furthermore, Mn^{2+} was found to inhibit catalysis in the presence of magnesium but the absence of spermidine. An explanation for these metal ion dependence results is that only Mg^{2+}, Sr^{2+} and Ca^{2+} are capable of folding the RNA into a catalytically competent structure, although this is possible in the presence of spermidine with Mn^{2+} and Co^{2+}. Other divalent metal ions, monovalent metal ions and Mn^{2+} and Co^{2+} in the absence of spermidine could force the structure into a catalytically incompetent conformation that does not permit association of the two loops 1 and 2.

The hairpin ribozyme is thus an example of a new class of ribozymes, which, although metal dependent, could be termed nonmetalloenzymes in the sense that metal ions play a passive role in catalysis.

VS ribozyme

The *Neurospora* VS ribozyme is a small catalytic RNA motif capable of self-cleavage and ligation *in vivo* and *in vitro*. It is unusual in that the substrate for the *in vitro trans* cleavage is a stable hairpin, indicating that, unlike most ribozymes, substrate recognition is achieved through tertiary interactions rather than through Watson–Crick base-pairing. It is thought that there is a great deal of tertiary structure in the VS ribozyme.

Four metal-binding sites have been identified by ethylation protection and modification interference experiments. There are four phosphate groups implicated in direct metal ion binding which are probably located at the core of the ribozyme. There are two clusters; the first lies within the internal loop that contains the cleavage site. The second occurs in helix VI.

HDV ribozyme

The structure of HDV revealed a novel double-pseudoknot fold and a lack of catalytic divalent metal ions. Recent work has shown that imidazole buffer is capable of rescuing cleavage activity of an HDV ribozyme in which the 'active' cytosine has been changed to a uracil (Perotta *et al*., 1999). This suggests that imidazole and, therefore, the original cytosine serve as the general base catalyst in the catalyst in the self-cleaving reaction, thus arguing that it is the RNA itself, rather than metal ions, which directly initiate catalysis.

Group I intron

The large ribozymes (group I introns, group II introns and RNase P) are all thought to utilize the two-divalent-metal-ion mechanism. In this model, one ion activates the nucleophile whilst another coordinates the leaving group, stabilizing the transition state.

The *Tetrahymena thermophila* group I intron performs a two-step transesterification reaction using a single active site (Cate *et al*., 1996). The latest evidence on the mode of catalysis supports a minimum of three metals playing a role. One metal acts as a base that activates the 3′-hydroxyl nucleophile, another acts as an acid coordinated to the leaving group. The third metal might help in the activation of the nucleophile by interacting with the 2′-hydroxyl of the guanosine cofactor. Whilst a fourth metal may be expected to help stabilize this complex, that role is actually performed by a conserved nucleotide triad. The group I intron seems be a true metalloenzyme, specifically binding ions that play roles required for catalysis.

RNase P

Although the RNase P reaction absolutely requires divalent metal ions (preferably Mg^{2+}) for catalysis, tRNA can bind to the RNase P RNAs of *Bacillus subtilis*, *Escherichia coli* and *Chromatium vinosum* in the absence of metal ions (Oh *et al*., 1998). Although, Mg^{2+} is not required for substrate binding, the efficiency at which crosslinked species are obtained is notably improved (about 7- to 10-fold) by the addition of Mg^{2+} in the case of the *B. subtilis* RNase P RNA. It has also been reported that the folding of RNase P RNA is cooperatively dependent on Mg^{2+} concentration and that at least three Mg^{2+} ions contribute to folding.

In contrast to their contribution to ribozyme folding and substrate binding, divalent metal ions, preferably Mg^{2+}, are absolutely required for catalysis. Maximal catalytic activity requires multiple metal ions (Hill coefficient = 3.2 for *E. coli* RNase P RNA). Mn^{2+} can substitute for Mg^{2+} with only a slight decrease in catalytic efficiency, whereas Ca^{2+} results in a 10^4-fold reduction in k_2. No other metals are known to stimulate RNase P activity. The high degree of specificity that RNase P exhibits for catalytic metal ions suggests that the ribozyme–substrate complex forms a highly ordered metal-binding pocket that constitutes a portion of the active site. Given the ability of divalent metal ions to lower the pK_a of bound water molecules, it is likely that at least one of the metal ions that are required for catalysis serves to direct an attacking nucleophilic hydroxide ion into the scissile phosphodiester bond.

RNA Folding

Metal ions in secondary structure

The crystal structure of the P4–P6 domain of the *T. thermophila* group I intron revealed major groove binding sites for Mg^{2+} at tandem G•U base-pairs (Cate *et al*., 1996). A G•U base-pair has only hydrogen bond acceptors in the major groove, compared with the G-C or A-U base-pairs that only have hydrogen bond donors in the major groove. NMR studies have estimated the K_d of Mg^{2+} binding to tandem G•U base-pairs to be in the millimolar range.

The G•U binding sites have also been observed in the P1 helix, P5 helix and the P5b stem–loop of *T. thermophila*. They are preformed for the acceptance of a metal ion. However, they are all present near tertiary interactions in the ribozyme. It is hypothesized that they are secondary structure sites that facilitate tertiary structure formation. The bound metal ions reduce the electrostatic repulsion of the phosphate groups from the different secondary structure elements that must come together in the tertiary structure.

Based on this study the conclusion is that divalent metal ions bind to preformed sites in the secondary structure of RNA and that these metals are not required for secondary structure formation, but are essential for the formation of the tertiary structure.

The structure of loop E fragment of 5S RNA revealed a number of Mg^{2+} ions that line the major groove of the helical fragment (Correll *et al*., 1997). Five ions lie along the groove in total and help to pull the backbones to a very narrow 6-Å separation between the phosphate atoms. The Mg^{2+} lie about 2.7 Å apart and are bridged by three water molecules.

The minimal human immunodeficiency virus type 1 (HIV-1) RNA dimerization initiation site is a 22-base pair duplex with two noncanonical Watson–Crick-like G-A mismatches each adjacent to a bulged adenosine. The crystal structure revealed a network of eight magnesium ions. Five of the magnesium ions are fully hydrated and lie in the deep groove and are coordinated to the Hoogsteen sites of guanine nucleotides. Three further magnesium ions make up a motif called a ‘magnesium clamp’. Two ions make direct contact to phosphate oxygens in regions containing bulged adenosine nucleotides and the third magnesium directly bridges, across the deep groove, the two phosphates 3′ to each bulge.

Tertiary structure

Some metal ion-binding sites are formed only after or during tertiary structure formation. This is seen in the folding of the RNA pseudoknot, when a single strand at the end of a stem–loop folds back to form the pseudoknot, forming a metal-binding site. This does not mean that the pseudoknot forms only in the presence of the divalent metal ion; high concentrations of univalent metal ions can have a similar effect. An analogous binding site is formed when two hairpin loops pair to form kissing hairpins. Mg^{2+} is known to bind upon complex formation but the exact binding site has not been determined. Complete base-pairing of the two loops requires tight turns in the loops

Figure 3 The crystal structure of the P5–P6 domain of the *Tetrahymena thermophila* group I intron showing the magnesium core. (The magenta sites are cobalt hexamine sites in the crystal structure, which are analogous to hexahydrated magnesium.)

that bring together several phosphates, probably producing a metal-binding site.

The crystal structure of the P4–P6 domain has revealed a wide range of tertiary metal ion-binding sites (**Figure 3**) (Cate *et al*., 1996). Mg^{2+} knit together the tertiary structure of the P4–P6 domain and in so doing change the secondary structure. In total there are 12 Mg^{2+} sites in the P4–P6 domain. Two Mg^{2+} bind to phosphate groups inside the A-rich bulge in the P5abc, turning the bulge inside out so that the bases are on the outside interacting with the stem of the P4 region. A guanine from the bulge is displaced in the tertiary structure and binds via Mg^{2+} to the P5c stem. Two G•A base mismatches in close proximity also bind a further two Mg^{2+}. These metal ions are called a magnesium core, whose formation is necessary for the formation of the tertiary folding of the P4–P6 domain. These sites are only present in when the tertiary structure folds. Without the metal ions neither the site nor the tertiary structure exists.

Biochemical analysis of the whole *Tetrahymena* group I intron led two groups independently to reach the conclusion that there is an optimum magnesium concentration for proper folding. Too little magnesium and the structural metal sites will not be occupied, while too much magnesium may inhibit the folding process. The optimum amount for correct folding is just above that required for structure formation. The explanation for this may be that excess magnesium can cause the structure to fall into kinetically stable intermediates which block further folding. Alternatively, multiple folding pathways may exist and factors such as temperature, RNA sequence and the ion concentration may all play a role in determining the final structure.

Thermodynamics

Our current understanding of Mg^{2+} binding to RNA, in both thermodynamic and structural terms, is largely based on classical studies of tRNAs. Based on these studies, it is clear that magnesium ions are crucial for stabilizing the folded structure of tRNA. It was initially shown that Mg^{2+} and later other divalent and some univalent metal ions could restore the amino acid acceptor activity of denatured tRNA. Mg^{2+}, however, stabilizes the native tertiary structure of tRNA at a physiological concentration.

The thermodynamic and structural data have been used to suggest that a small number of Mg ions strongly bound to a few specific sites are crucial in stabilizing the tertiary structure of tRNA. Much less attention has been given to the many more weakly bound ions that must also play a key role in stabilizing the RNA. This omission probably arises from the difficulty in modelling the electrostatic forces that dominate the interactions of these nonspecifically bound ions. Unfortunately, the long-range electrostatic interactions governing Me^{2+} binding cannot be quantitatively described by the simple mass action equilibria inherent in the analysis of binding polynomials. Therefore, these analyses provide no more than a purely phenomenological view of magnesium binding to RNA with little insight into the mechanism of the binding equilibria. In the absence of a rigorous model for RNA–magnesium interactions, one must ask if a small number of 'strong binding sites' are necessary to explain the observed thermodynamic properties at all. In fact, even semi-quantitative electrostatic models of Mg^{2+} binding to tRNA lead to very different interpretations of Mg^{2+} binding isotherms compared with standard binding polynomials. Thermodynamic descriptions of Mg^{2+} binding to tRNA have been based on the analysis of binding polynomials to estimate the energetics and stoichiometry of different classes of bound ions.

Recent investigations propose that the nonlinear Poisson–Boltzmann (NLPB) equation provide a remarkably accurate description of both the overall stoichiometry and the free energy of Mg^{2+} binding to yeast $tRNA^{phe}$, without any fitted parameters. A thermodynamic description of Mg^{2+} binding to yeast $tRNA^{phe}$ can be based upon the NLPB equation. Mg^{2+} binding is simply explained by an ensemble of ions distributed according to a Boltzmann weighted average of the mean electrostatic potential around the RNA. It appears that the entire ensemble of electrostatically bound ions superficially mimics a few

strongly coordinated ions. In this regard, we find that Mg^{2+} stabilizes the tertiary structure of yeast $tRNA^{phe}$ in part by accumulating in regions of high negative electrostatic potential. These regions of Mg^{2+} localization correspond to bound ions that are observed in the X-ray crystallographic structures of yeast $tRNA^{phe}$. Based on these results and the available thermodynamic data, there is no evidence that specifically coordinated Mg ions have a significant role in stabilizing the native tertiary structure of yeast $tRNA^{phe}$ in solution.

Conclusions

The role of divalent metal ions in RNA structure and function is clearly unresolved and under intensive study. In the last decade there has been an explosion of RNA structural information from X-ray crystallography and NMR studies. Many of the crystal structures have identified tightly bound divalent cations complexed to the RNA directly or through the water shell of the metal. Even more recently, NMR has been used to locate bound divalent metal ions by chemical shifts induced by manganese or cobalt hexammine. These structural studies have allowed determination of the binding preferences of the metals and conformational motifs assumed by RNA bound to divalent cations.

Some of the important outstanding questions include: (1) Does divalent metal ion binding induce a conformational change in RNA or is the binding site preformed? (2) Are divalent cations directly involved in catalysis of ribozymes or do they serve a structural function? (3) What is the relative contribution of electrostatics versus specific coordination in the high-affinity metal-binding sites identified by crystallographic studies? (4) Are there common RNA metal-binding motifs that can be used in RNA engineering efforts?

The studies described in this review are directed toward answering these questions. For example, NMR studies show that conformational changes occurring on metal ion binding appear to maintain secondary structure while promoting tertiary structure formation. Theoretical studies suggest that most divalent metal-binding sites are electrostatic in nature and that specific bonds formed by the metal ion or its water shell are secondary in nature. Crystallographic studies have not yet found metal ions directly responsible for cleavage reactions in ribozymes, suggesting, along with some biochemical studies, that the RNA itself may be performing the cleavage reaction with the metal ions stabilizing the proper RNA conformation.

Technological and methodological advances may ultimately answer these questions. For example, the recent determination of the crystal structure of the large and small ribosomal subunits, including the 5S, 16S and 23S ribosomal RNAs, greatly increases our database of RNA structure and associated divalent cations. Further thermodynamic, kinetic and theoretical studies will be important in understanding and interpreting the rapidly expanding structural database.

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